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Micro propagation studies of *Albizia amara (Roxb.) Boiv.* through axillary bud culture

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Abstract
Albizia amara, belonging to the family Fabaceae is a valuable economic, medicinal and multipurpose drought tolerant tree commonly found in dry forests of India. Keeping view of its economic importance, a protocol was developed for rapid clonal multiplication of *Albizia amara* by means of plant tissue culture. The present study describes a protocol for rapid *in vitro* micropropagation of *Albizia amara* during through nodal segments containing axillary buds. The buds induced to produce a large number of multiple shoots by culturing on MS medium supplemented with different concentrations of BA (benzyladenine) and NAA (α-naphthalene acetic acid). The maximum number of shoots per explants was obtained on MS medium supplemented with 1.0 mg/L BA and 0.1 mg/L NAA was after 8 weeks of culture. Excised shoots were rooted on half strength MS medium fortified with 0.5 mg/L either IBA (Indolbutyric acid) or NAA alone. The complete plantlets thus obtained were successfully transferred to soil.

Keywords: *Albizia amara*, tissue culture, *in vitro*, plant regeneration

Introduction
Albizia amara (Roxb.) Boiv., belonging to the family Fabaceae, is a valuable economic, medicinal and multipurpose drought tolerant tree commonly found in dry forests of India. The wood of *Albizia amara* is purplish brown with lighter bands, very hard and strong, used for cabinets in building and agriculture purpose. The bark of the tree yields gum, which is used for ulcers (Kashyapa and Ramesh, 1992) [1] and molluscidal activity (Ayoub and Yankov, 1986) [2]. Besides these, the leaves contain a flavanol glycoside namely 4'O–menthylrutin and they are extensively used as herbal cosmetic for hair maintenance. Leaves are also useful in ophthalmia. *Budmunchiamines*, spermine macrocyclic alkaloid (Mar *et al*., 1991) [3] extracted from the seeds of *A. amara* were found to interact with DNA by inhibiting the catalytic activity of DNA polymerase, RNA Polymerase, and HIV reverse transcriptase. *Albizia* is also one among the 10 major species, which is widely used in plantation forestry programme in India. (Kumar *et al*. 1998) [4] described regeneration of plants from leaflet explants in *A. procera.* (Sinha *et al*. 2000) [5] described *in vitro* differentiation and plant regeneration in *A. chinensis. (*Ramamurthy *et al.* 2003) [6] described shoot bud regeneration from leaf explants. Micropropagation via axillary bud proliferation was not described in *A. amara*, hence this study was undertaken. This paper describes a reproducible and efficient protocol to regenerate true to type plantlets directly from seedling explants.

Material and Methods
Shoot apices having nodal segments with axillary buds were collected from natural trees growing in my living area. The buds were disinfected with 0.5% sodium hypochlorite solution with 3-5 drops of Tween- 20 for 20 minutes and washed thoroughly with tap water. The buds were also treated with 0.05% mecuric chloride solution for 5 minutes then washed with sterile distilled water and pre-treated with citric acid (150 mg/l), ascorbic acid (100 mg/l). The sterilized nodal explants were transferred to (Murashige and Skoog. 1962) [7] MS medium supplemented with growth regulators at various concentrations and combinations of BA with NAA for shoot induction. Excised shoots from these cultures were rooted on ½ MS supplemented with IBA (or NAA at different concentrations for root induction pH was adjusted to 5.6 using 0.1 N NaOH or 0.1 N HCl before autoclaving (121°C under 1.05 kg/cm2, 20 min). The cultures were maintained in a culture room at 25±2°C and were exposed to continuous fluorescent light for 16 h per day and successfully transferred to soil. Significant differences among mean values were using One-way statistic method, multiple-range test was conducted to evaluate differences among the treatments.

Results and Discussions
Micropropagation is the true-to-type propagation of selected cultivar using *in vitro* technique. The segments of *Albizia amara* exhibited browning of the explant and medium due to leaching of phenolics. This phenomenon results from physiological changes within the cultured tissues that lead to gradual browning and eventual death of tissues. (Alkhateeb. *et al.* 2002) [8] T issue browning is a problem frequently observed during *in vitro* establishment of explants from woody plants (Block, R *et al*. 1996) [9]. The problem of phenolic browning was minimized to a great extent by leaching of phenolic compounds due to agitation in antioxidants solution and by proper drying of explants prior to inoculation (Meghwal, P. *et al*. (2001) [10] Significantly reduced leach in by supplementing the medium with citric acid (150 mg/l), ascorbic acid (100 mg/l) This agreed with (Badawy, A. 2005) [11]. The variance analysis of the axillary bud cultures showed that the effects of the treatments with

different combination of BA and NAA were significant on number of shoots. The nodal segments proved to be excellent explants for multiple shoot formation and the first response of axillary buds within two weeks. New shoot development from axillary bud was observed within three weeks of culture and more shoots were found to develop during subcultures. The best response was found under 1.0 BA+0.1 NAA mg /L (Table 1) combination which was found most effective (Fig. 1). The combined effects of BA+NAA on shoot induction were reported earlier in different plants. Our results agreed with those obtained by (Lal, N. *et al*. (2000), Munshi, M *et al*. (2004), Al-Sulaiman, M. *et al*. (2010) [12, 13, 14]. On the other hand, the adding of NAA in different concentrations developed the multiple shoots in this study but the best concentration was 0.6 mg/l. Root formation was induced when elongated shoots (1-2 cm) were transferred to ½ MS medium fortified with 0.5 mg/L IBA or NAA. The result showed that the IBA is considered as the most effective auxin in root induction. Elongated shoots derived from the explants were separated and cultured on half strength MS supplemented with IBA (0.5 mg/l) for induction of rooting. The optimum concentration was 0.5 mg/l ((Fig. 2)) of IBA and it resulted in 85% of root initiation within 1 2 weeks of culture. (Dhabhai, K, *et al*. (2010), Tomar, U (1988). [15, 16] The complete plantlets were transferred to small plastic pots, and bags containing a mixture of soil and compost then transferred to the field. (Fig. 3)

Table 1: Shoot regeneration from nodal explants Albizia on MS medium consisting of BAP+NAA after 8 weeks of culture

BAP+NAA conc.(mg/l)	No. of shoots mean	No. Of shoots ± SD	Mean Length of shoots(cm)	Length of shoot ± SD
05+0.1	1.4	1.14	0.48	0.54
0.75+0.1	2.2	1.30	0.4	0.70
1.0+0.1	5.8	1.92	0.88	1.14
1.25+0.2	2.8	1.30	0.64	0.83
1.50+0.25	2.4	0.54	0.48	0.54

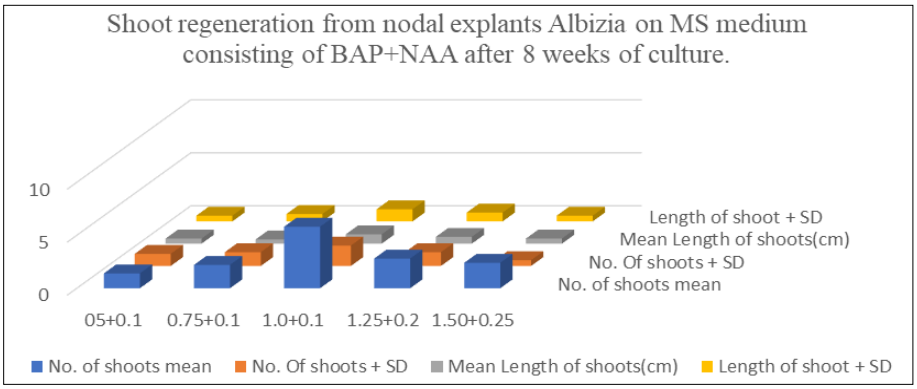

Fig 1: Shoot regeneration from nodal explants Albizia on MS medium consisting of BAP+NAA after 8 weeks of culture

Table 2: MS medium consisting of IBA/NAA for root development *in vitro* raised roots after 8 weeks of culture

Hormone conc.(mg/l) IBA/NAA	No. of roots mean	No. Of roots ± SD	Mean Length of roots(cm)	Length of root ± SD
0.10+.10	1.4	0.54	1.64	0.50
0.25+0.25	1.6	0.54	1.72	0.58
0.50+0.50	4.2	1.30	3.42	0.83
0.75+0.75	2.0	0.70	2.12	0.39
1.0+1.0	1.8	0.83	1.97	0.37

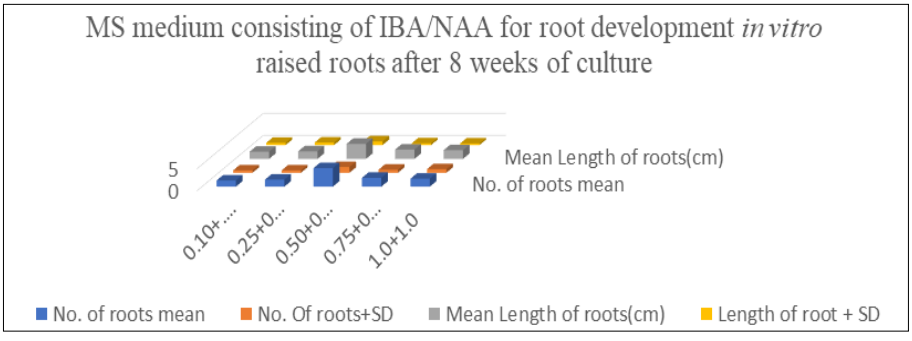

Fig 2: MS medium consisting of IBA/NAA for root development *in vitro* raised roots after 8 weeks of culture

Acclimatization of rooted plantlets

Acclimatization and hardening are the most important aspects for micropropagated tree species. *In vitro* hardening can be achieved by decreasing the water potential of the medium and reducing humidity in culture vessel. *In vitro* regenerated complete plantlets of *A. amara* were taken out

of the rooting media and agar traces were removed by washing with distilled water for10 to 15 min. Fungal growth rate was checked by spraying 0.5% (w/v) Benolate fungicide. Then plantlets were transferred to pots of size 7.6 cm^2 containing sterile peat moss and vermiculate (1:1) and incubated for two weeks at 25 □ 2°C (2000 lux) for 16 h photoperiod. The pots were enclosed in plastic covers and small holes were punched to decrease the relative humidity. During the period, liquid quarter strength MS basal nutrient medium was provided. Gradually, pots were transferred to room temperature having diffuse light. Later, pots were shifted to green house and size of the holes was increased. Covers were removed after 6 weeks and plants were acclimatized in normal soil (Figure 4). About eight to nine true-to-type plantlets of *A. amara* were raised *in vitro* from single axillary bud explant. But successful transfer of *in vitro* raised plantlets to soil through hardening is poor (50%) due to premature defoliation of leaflets.

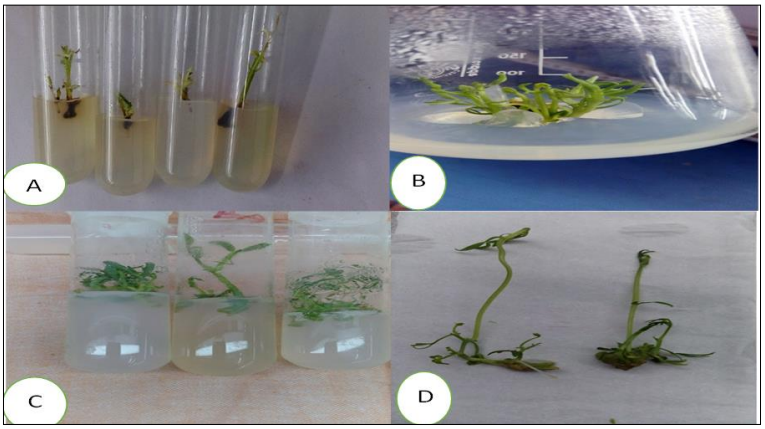

Fig 3: A. Inoculation of Axillary Bud of *Albizia amara.* B-C. Shoot regeneration from axillary bud explants, 15 days after inoculation, D. Multiple shoots, 45 days after inoculation

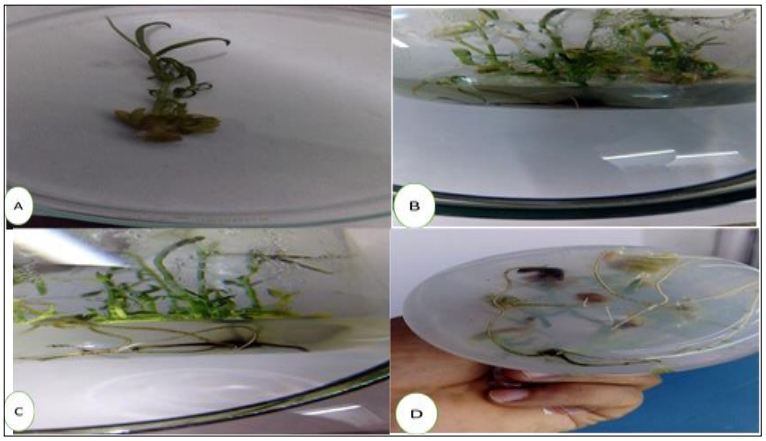

Fig 4: Initiation of roots from basal end of regenerated shoot on MS medium. Fig. (A) and (B) Further elongation of roots after 2 weeks. Fig. (C). Formation of 5- 7 roots on MS medium. Fig. (D). Formation of thick branched roots on MS medium with BAP/NAA (0.5/+0.05 mg/l). Further growth and elongation of roots

Fig 5: Plantlet transferred to poly bag and kept under growth room conditions

Conclusion

The present study successfully established an efficient and reproducible protocol for direct *in vitro* regeneration of *Albizia amara* using axillary bud explants, contributing to its conservation and large-scale micropropagation. Among the tested treatments, MS medium supplemented with BAP (1.0 mg/l) in combination with NAA (0.1 mg/l) proved optimal for inducing multiple shoot formation. Furthermore, effective *in vitro* rooting was achieved on half-strength MS medium fortified with IBA and NAA (0.50 mg/l each). This protocol offers a reliable system for rapid clonal multiplication and can play a significant role in the conservation and sustainable utilization of this valuable species.

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